Agronomic character and RAPD marker - ICSARD - Agam - FKS

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Agronomic Traits and RAPD Markers for Diversity Analysis in Soybean [*Glycine max* (L.) Merill] Mutant Using Gamma Rays at Saline Soil

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Abstract

Soybean is one of the strategic food commodities in Indonesia, but the production of soybean is not equally with the demand. It is occur due to lack of land for soybean cultivation by many factors. One of the way to meet the demand is by improving soybean cultivation on marginal land such as saline soil. Breeding soybean tolerance to saline soil is the key for solving the problem. The purpose of this research is to evaluate the genetic diversity in soybean mutants using gamma rays based on agronomic traits and RAPD markers. A total of 200 seeds of soybean were planted at saline soil with electrical conductivity 1,2 – 4,3 dS/m. Soybean cultivar Detam-3 was treated by gamma rays radiation at 0, 160, 208, 256, 304, 352, 400, 448, 496, 544, and 592 gy. Genetic analysis using RAPD markers was done on one plant for each treatment. OPAA-02 and OPAA-14 were used as RAPD markers. Agronomic marker was measured at plants surviving until harvesting time. The result showed that polymorphism among eleven plants were 60 % and 83.3 % based on OPAA-02 and OPAA-14 markers, respectively. There were 54 plants that can be survived in saline soil. The agronomic traits of 54 plants has high diversity.

Keywords: Detam-3, Gamma Rays, OPAA-02, OPAA-14, Saline Soil, Soybean.

Introduction

Soybean (*Glycine max* L. Merr.) is one of main commodities that are widely planted in many countries. Soybeans are used as food, feed, and raw materials of industrial product (Hartman et al. 2011). It has a relatively high protein content (35-46%) (Ginting et al. 2009). However, the soybean production is not equally to the demand. Domestic production was 963,18 thousand tons at 2015, while domestic consumption reached 1,56 million tons (Nuryati et al. 2016). The increased of domestic consumption is 1,73% each year (Ministry of Trade 2014). On the other hand, the land for soybean cultivation has decreased. The harvested area in 2009 was 722.291 ha, then it was decreased to 550.793 ha in 2013, while in 2015 the area was increased up to 614.095 ha (Prawito 2016). However, the domestic soybean production is unable to meet the soybean demand. Harsono (2008) estimated 2 million ha of cultivation area is needed to fulfill the demand in 2020.

Salinity becomes a global challenge in agricultural production (Wu 2018). Up to 80% of plant yield can be lost because of drought and salinity (D'Souza et al. 2009). One approach of using saline soil as cultivation land is through salt tolerance. First step of breeding program is creating genetic diversity through crossing

(Utomo, et al. 2018), exploration (Xu and Gai 2003), and mutation (Arwin 2015). Mutation breeding has been widely used for inducing variation, there were 2 type of mutation including chemical mutation using mutagen EMS (Luan et al. 2007), and physical mutation using gamma rays (Kusmiyati et al. 2018). Mutation breeding is considered more effective to improve traits and more efficient to screen new traits (Sobrizal 2016). RAPD technique as one alternative for identification plant genetic diversity (Doldi et al. 1997; Tidke et al. 2017).

This study was aimed to evaluate the genetic diversity in soybean mutants using gamma rays based on agronomic traits and RAPD markers. This research is a series of research to obtain adaptive mutant black soybean plant in saline field with better production compared to its parents in saline soil.

Materials and Methods

This research was conducted at Saline Soil, in Dresi Wetan, Kaliori, Rembang, Central Java, and Central Laboratory of Diponegoro University, Semarang, Central Java in December 2017 to July 2018. The cultivar of soybean was Detam-3. The treatment of gamma ray radiation were 0 (control), 160, 208, 258, 304, 352, 400, 448, 496, 544, and 592 Gy. Induced mutation was done at the Center for Applications of Isotopes and Radiation, National Nuclear Energy Agency (PATIR-BATAN). Two hundreds seeds were planted at saline soil with electrical conductivity (EC) 1,2 – 4,3 dS/m. The genotype was namely BSMG (Black Soybean Mutant Gamma).

Parameters measured were agronomic traits and molecular markers. The agronomic traits were, number of leaves, plant height, number of pods, average seed per pod, seed weight per plant, weight per unit of seed, and 100 seed weight. Molecular markers were obtained based on RAPD (Random Amplified Polymorphic DNA).

The leaf samples for molecular analysis were taken from one plant of each treatments at three weeks after planting. The stages of DNA extraction and isolation were carried out using Plant Genomic DNA Kit Tiangen. DNA amplification was performed using OPAA-02 and OPAA-14 markers, which were used as DNA markers of soybean genotypes under salt stress (Khan et al. 2013; Mahgoub et al. 2016). The DNA amplification stage is carried out in a reaction volume of 25 µl per sample.

The method of amplification DNA was modified method from Khan et al. (2013), PCR-mix was consist of 22 µl Master Mix (12,5 µl AmpliTag Gold 360, 1 µl 360 GC Enhancer, 8,5 µl ddH_2O), 1 µl Primer (working solution 25 µM), and 2 µl DNA Template. The amplification reaction was carried out in a controlled Thermal Cycler (Labnet, MultiGene OptiMAX). The first cycle consisted of denaturation of template DNA at 95 ºC for 10 min. Followed by Primer that used annealing stage require temperature at 37 ºC for 30 sec, and primer extension require temperature at 72 ºC for 2 min. In the next 43 cycle, the period of denaturation was reduced to 30 sec while the stage annealing and extension time remained as in the first cycle. The last stage was final extension at 72 ºC for 8 min.

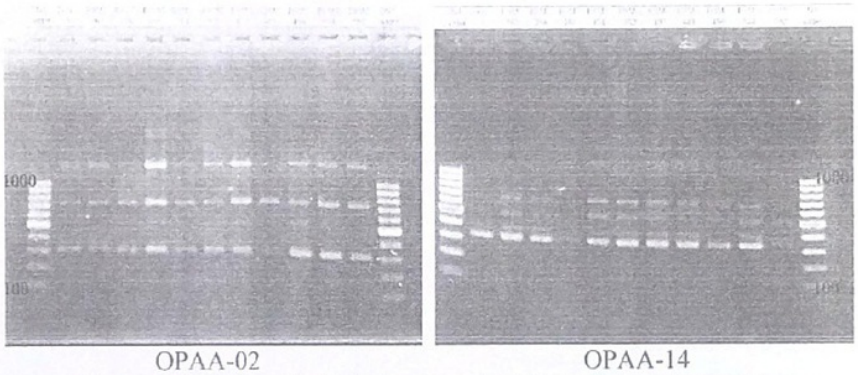
Product of PCR were separated on a 1,5 % agarose gel and DNA Fragment were visualized using GelDoc.

Analysis of agronomic parameter was done by analysis of variance (ANOVA), at 5% significance level, followed by Dunnet test to compare each treatment to control plant (Detam-3). The RAPD products were scored as present (1) or absent (0) for each primer. The data entry was done into a binary data matrix as discrete variables. This matrix was subjected to unweighted pair-group method for arithmetic average analysis (UPGMA) to generate a dendogram using average linkage procedure. All these computations were carried out using NTSYSpc Software.

Result and Discussion

Molecular Parameters

Based on a RAPD Analysis that using OPAA-02 and OPAA-14 (**Fig. 1**), there were 5 fragments with polymorphic (60 %) and 6 fragments with polymorphic (83.3 %), respectively (**Table 1**).



OPAA-02 OPAA-14

Figure 1. The Result of Electrophoresis OPAA-02 and OPAA-14

Table 1. Primer and Number of Fragments of Marker Amplification OPAA-02 and OPAA-14.

Primer	Sequence	Fragment Size	Total Fragment	Polymorphic Fragment	% Polymorphic
OPAA-02	GAGACCAGAC	300 – 2220	5	3	60
OPAA-14	AACGGGCCAA	300 – 1210	6	5	83.3

The result showed that there were appearance of new bands occurred in comparison to the controls (Detam-3) on the OPAA-02 marker at 2220 bp (BSMG-256) and 520 bp (BSMG-256, 352, 400, 496, and 544). As well as on the OPAA-14, there were new bands that do not appear on Detam-3 but appear on the other genotypes, which were 1210 bp (BSMG-304, 400, and 448), and 850 bp (BSMG-544). Meanwhile, there was a band which disappear in comparison to the control in 1340 bp on BSMG-448 at OPAA-02 marker. On the OPAA-14 marker also showed that there were some bands disappear on BSMG-256 (700, 520, and 370 bp). Based on these differences indicate the occurrence of genetic mutations that can cause changes in certain traits (**Table 2**).

Table 2. Analysis Bands of OPAA-02 and OPAA – 14

Primer Name	Fragment of Bands (bp)	Detam 3	Genotype									
			160	208	256	304	352	400	448	496	544	592
OPAA-02	2220				+							
	1340	+	+	+	+	+	+	+	-	+	+	+
	740	+	+	+	+	+	+	+	+	+	+	+
	520				+		+	+		+	+	
	300	+	+	+	+	+	+	+	+	+	+	+
OPAA-14	1210					+		+	+			
	850										+	
	700	+	+	+	-	+	+	+	+	+	+	+
	520	+	+	+	-	+	+	+	+	-	+	+
	370		+	+		+	+	+	+	+	+	
	300	+	+	+	+	+	+	+	+	+	+	+

The genetic diversity was visualized by RAPD analysis, the result showed the diversity of bands in RAPD profiles. It showed the genetic variation among control and gamma rays treated. Juwarno and Samiyarsih (2017) reported that the salt stresses condition of three soybean cultivars (Mahameru, Slamet, Dam) showed that there were genetic differences between control plot and 80 mM NaCl plot. Mahgoub et al. (2016) reported that genetic of soybean showed instability caused by salinity-stress, it was proven based on RAPD profiles changes through the decrease or the increase in band intensity, disappearance of bands, and appearance of new bands occurred in the profiles in comparison to the controls.

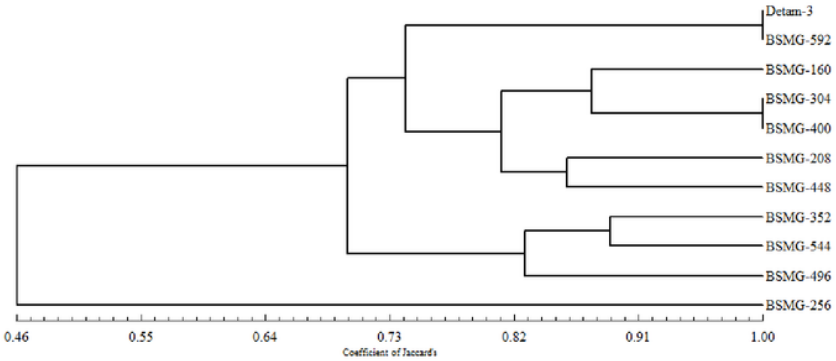


Figure 2. Dendrogram derived from UPGMA clustering analysis based on Genetic Different in Soybean Mutant

Based on the dendogram chart, it indicated that the genotypes were clustered in four groups. The smaller value of the Jaccard coefficient indicates the farther kinship relationship. The jaccard coefficient value which ranges from 1.00 to 0.70. First cluster comprising control, mutant BSMG-592 (100% similarity). Second cluster included BSMG-160, 208, 304, 400, and 448 (74% similarity). Third cluster included BSMG-352, 496, and 544 (70% similarity). The highest genetic distance from control (Detam-3) was BSMG-256 (46% similarity). These conditions showed that there was diversity from control to the other genotypes that indicates mutation activity influenced the genetic diversity of soybean plants. These results were in compliance with the other researchers (Zainudin et al. 2014) who reported that the

diversity analysis could be used by RAPD analysis and the genetic distance was measured by jaccard coefficient. According to Arisetianingsih et al. (2010), kinship is a relationship between the closeness of a species based on phenotype and genotype. Therefore, if a species has similarities with each other it can be suspected to have a close kinship.

Agronomic Parameters

Growth Traits

Based on the analysis of variance, gamma ray radiation affected number of leaves and plant height. The Dunnet-Test was showed at **Table 3**.

Table 3. Number of Crops, Mean for Number of Leaves and Plant Height of Black Soybean in Saline Soil.

Genotype	Number of Crops	Number of Leaves	Plant Height
BSMG – 160	7	5.33 ± 1.16	8.67 ± 0.76*
BSMG – 208	6	13.33 ± 1.53	8.50 ± 3.12*
BSMG – 256	3	8.67 ± 0.58	14.00 ± 2.00
BSMG – 304	8	24.00 ± 18.25*	7.33 ± 1.89*
BSMG – 352	3	10.67 ± 1.15	9.33 ± 2.31
BSMG – 400	5	28.00 ± 10.15*	9.83 ± 2.75
BSMG – 448	3	13.00 ± 3.46	11.50 ± 2.78
BSMG – 496	3	9.33 ± 3.51	12.17 ± 4.75
BSMG – 544	0	0.00 ± 0.00	0.00 ± 0.00*
BSMG – 592	4	19.00 ± 6.08*	18.33 ± 7.50
Detam – 3	12	6.00 ± 5.20	14.33 ± 2.08
CV (%)		46.74	28.88

Notes : The Value with * has significant difference than the control (Detam-3) based on Dunnet-Test at $P \leq 0.05$; CV= Coefficient of Variation.

Leaf Numbers of genotype BSMG-304, 400, and 592 were significantly different with Detam-3 in saline soil. Plant at treatment of BSMG-304, 400, and 592 had an average number of leaves of 24, 28, and 19 leaves, respectively. In addition, the plant height of BSMG-160, 208, 304, and 544 were significantly different from Detam-3. Plant height of Detam-3 was 14.33 cm, meanwhile plant height of BSMG-160, 208, and 304 were 8.67 cm, 8.50 cm, and 7.33 cm, respectively. Balitkabi (2016) reported that plant height of Detam-3 was approximately 56.9 cm under optimum condition. Plant height at saline soil in this research was lower. The decreased of plant height was caused by the effect of salinity-stress that can inhibit growth, and the influence of gamma ray radiation that can cause mutation in plant traits, such as dwarfing. These results were in compliance with the other researchers who reported that the decrease in plant height was due to the effect of salinity-stresses of plant growth (Phang et al. 2008), or the effect of gamma ray radiation (D'Souza et al. 2009).

These results have been confirmed by Yuniati (2004), that the plant which affected by salinity-stress will show a delayed growth responses like a decreased of plant height, leaf area, dried apical buds, and even death on plant. The number of leaves on the plant influenced the rate of photosynthesis. According to Qados (2011), salinity-stress in high concentration of NaCl decreased plant height, number

of leaves, and leaf area on bean plant, due to the negative effects of ions Na^+ and Cl^- on the rate of photosynthesis, changes in enzyme activity, and also decreased levels of carbohydrates and growth hormones which can cause growth inhibition. On the other hand, D'Souza et al. (2009) stated gamma ray radiation in soybean can cause mutations in plant traits, such as dwarfing. Based on research conducted by Kusmiyati et al. (2018), gamma ray radiation in soybeans can affect the length, width, and density of stomata that influence the process of respiration and photosynthesis.

Production Traits

Based on the analysis of variance, it showed that the number of pods were not significantly different (P>0.05). Meanwhile, the seed per pod (BSMG-256, 352, and 544) and seeds weight per plant (BSMG-208) were significantly different to control (P>0.05) (**Table 4**).

Table 4. Mean for Number of Pods, Average of Seed per Pod, Seeds Weight per Plant, Weight per Seed, and Weight of 100 Seeds of Black Soybean in Saline Soil.

Genotype	Number of Pods	Average of Seed/Pods	Seeds Weight/Plant	Weight/Seed	Weight of 100 Seeds
BSMG – 160	2.00 ± 1.73	1,75 ± 0,43	0,17 ± 1,07	0,06 ± 0,01	5,77 ± 1,53
BSMG – 208	6.67 ± 2.89	1,67 ± 0,61	1,15 ± 0,54*	0,11 ± 0,03	10,88 ± 3,49
BSMG – 256	6.00 ± 3.46	1,88 ± 0,13*	0,67 ± 0,36	0,06 ± 0,03	6,30 ± 3,32
BSMG – 304	4.33 ± 1.15	1,53 ± 0,46	0,58 ± 0,26	0,10 ± 0,05	9,61 ± 4,60
BSMG – 352	2.00 ± 0.00	1,83 ± 0,29*	0,30 ± 0,02	0,08 ± 0,01	8,27 ± 1,11
BSMG – 400	5.00 ± 1.00	1,39 ± 0,18	0,73 ± 0,10	0,11 ± 0,03	10,88 ± 2,83
BSMG – 448	4.67 ± 3.79	1,59 ± 0,36	0,79 ± 0,74	0,10 ± 0,02	10,29 ± 2,09
BSMG – 496	4.00 ± 2.00	1,50 ± 0,50	0,40 ± 0,18	0,07 ± 0,02	7,38 ± 2,17
BSMG – 544	0.00 ± 0.00	0,00 ± 0,00*	0,00 ± 0,00	0,00 ± 0,00*	0,00 ± 0,00*
BSMG – 592	3.33 ± 3.21	1,16 ± 0,29	0,43 ± 0,31	0,13 ± 0,02*	12,67 ± 1,77*
Detam – 3	4.00 ± 1.00	1,30 ± 0,26	0,43 ± 0,19	0,08 ± 0,01	7,95 ± 1,42
CV (%)	47,96	20,79	53,28	25,21	25,21

Notes : The Value with * has significant difference than the control (Detam-3) based on Dunnet-Test at $P \leq 0.05$; CV= Coefficient of Variation.

It showed that each genotype was able to produce the same number of pods under salinity-stress conditions. Based on this research, the average number of pods ranged from 2.00 to 6.67 pods. However, according to variety description of soybean that was released by Balitkabi (2016), the trait of number pods per plant on variety Detam-3 in optimum condition can be reach approximately 51 pods per plant. It showed that the effect of saline-stress had decreased up to 96% of pods number. According to Yunita et al. (2018), with their study on Dering-1 at condition saline-stressed from several levels of salinity including 3 dS/m, 6 dS/m, and 9 dS/m decreased the number of pods in 32.7 %, 68.03 %, and 98.10 %, respectively.

Based on **Table 4** the traits of weight per seed and weight per 100 seeds were significantly different to control in BSMG-544 and 592. The weight per seed in BSMG-592 was higher than the other genotypes which linearly correlated with weight per 100 seeds trait. BSMG-592 had 0.13 g of weight per seed and 12.67 g of weight per 100 seeds, that classified to medium-size. According to Adie and

Krisnawati (2007) and Putra et al. (2015), the size of the soybean seeds are grouped into small (<10 g/100 seeds), medium (10-14 g/100 seeds), and large sizes (>14 g/100 seeds). The weight per 100 seeds of this result was higher (12.67 g) than Balitkabi (2016), reported that the weight per 100 seeds of Detam-3 in optimum condition can reach approximately 11.8 g. According to Putra et al. (2015), the purpose of plant breeding in the selection of genotype is based on the weight of seeds per plant and the weight of 100 seeds per plant to increase the production. Therefore, the radiation gamma ray with dose 592 Gy can gives a positive response on the weight of 100 seeds.

Conclusion

Based on a RAPD Analysis using OPAA-02 and OPAA-14, there were 5 fragments with polymorphic (60 %) and 6 fragments with polymorphic (83.3 %), respectively. Based on dendogram chart, the highest genetic distance from control (Detam-3) was BSMG-256 (46% similarity). BSMG-304 had significantly different than control (Detam-3) based on growth traits. Based on weight per seed and weight of 100 seeds, BSMG-544 and 592 had significantly different with control.

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1	R. K. Singh. "Identification of genetically diverse genotypes for photoperiod insensitivity in soybean using RAPD markers", Physiology and Molecular Biology of Plants, 10/2008 Publication	3%
2	Submitted to Universitas Diponegoro Student Paper	3%

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Agronomic character and RAPD marker - ICSARD - Agam - FKS

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